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THE MORPHOLOGY OF *NIPPOSTRONGYLUS MAGNUS*, A PARASITE OF NATIVE AUSTRALIAN RODENTS

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Summary

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Nippostrongylus magnus (Mawson) (Nematoda: Trichostrongyloidea) is redescribed from specimens from naturally-infected *Rattus fuscipes* from Blackwood, Victoria and from experimentally infected *R. fuscipes* and *R. norvegicus*. The asymmetry of the bursa, a characteristic of the genus, is matched by asymmetry of the spicules and genital cone. The synlophe is similar to that of *N. brasiliensis* but includes some variable features which appear to be of specific value. The morphological differences in *N. magnus* are discussed in relationship to the estimated period of separation from its congener, *N. brasiliensis*.

KEY WORDS: Nematoda, Trichostrongyloidea, rodents, *Nippostrongylus*

Introduction

During a study of the helminth parasites of the bush rat, *Rattus fuscipes*, a particular nematode species, *Nippostrongylus magnus* (Mawson) was encountered commonly in the duodenum. The species was described originally by Mawson (1961), although features of the complement of cuticular ridges, the synlophe, were not described. Some features of its synlophe were described by Durette-Desset (1969) and by Lichtenfels (1974), based on a small number of specimens, and in the latter paper their use for taxonomic purposes at the species level was considered. *N. magnus* has never been described in detail, and the use of the synlophe to identify species of *Nippostrongylus* as suggested by Durette-Desset (1970) and Lichtenfels (1974) has not been fully explored.

It was evident therefore that a detailed redescription of the nematode, particularly features of the synlophe, would allow a more definitive assessment of whether it provided useful taxonomic characteristics at the species level, as is the case in other trichostrongyloid genera. It would also provide a basis for subsequent ultrastructural and life-history studies of this parasite.

Materials and Methods

Nematodes were obtained from naturally infected *Rattus fuscipes* collected at Blackwood, Victoria (37°29'S, 144°19'E) and from laboratory raised *R. fuscipes* and *R. norvegicus* which had been infected experimentally with third-stage larvae of the species. Nematodes were collected live, washed in 0.9% saline and fixed in hot 70% ethanol. Additional specimens were fixed in 2.5% glutaraldelyde in phosphate buffer at 4°C and embedded in resin. Sections 1 μm thick were stained with toluidine blue, examined under the light microscope and photographed. Whole specimens were examined using Nomarski interference contrast microscopy after clearing in lactophenol and drawings were made with the aid of a drawing tube attached to an Olympus BH microscope. Apical views and transverse sections of the nematode body were prepared by hand using a cataract scalpel. Morphological terms for the complement of body ridges or synlophe and the numbering system for bursal rays follow Durette-Desset (1971, 1985).

Numbering of synlophe ridges was based on relationship to the axis of orientation of the synlophe as described by Durette-Desset (1971). Ridges dorsal to the axis were numbered from left to right 1, 2, 3 etc.; ridges ventral to the axis were numbered from left to right 1', 2', 3', . . . etc. Measurements are given in mm as the range followed by the mean of five specimens in parentheses.

Specimens examined have been deposited in the South Australian Museum (SAM), Adelaide.

Nippostrongylus magnus (Mawson)
FIGS 1-20

Austrohelgmonema magnum Mawson, 1961, pp. 816-817, figs 46-47, from *Rattus fuscipes*, *R. rattus*, *R. conatus*, *R. norvegicus* and *Melomys cervinipes*; Durette-Desset (1969), p. 737, fig. 3 (as *A. magna* from *Rattus* sp.).

Nippostrongylus magnus: Durette-Desset (1971), p. 818; Lichtenfels (1974), p. 286, (as *N. magna*); Obendorf (1979), p. 868, 896.

Material examined: From *Rattus fuscipes*: natural infections: 20♂♂, 20♀♀, Blackwood, Vic., 3♂♂, 4♀♀, deposited (SAM HC22877); experimental infections: 4♂♂, 2♀♀ (SAM HC22878); From *Rattus norvegicus*: experimental infections: 20♂♂ 16♀♀ (SAM HC22875).

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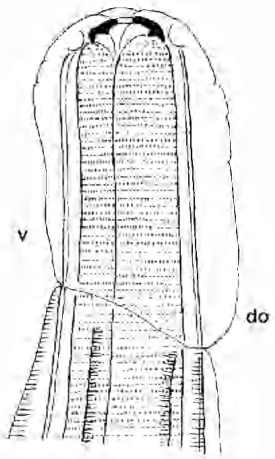

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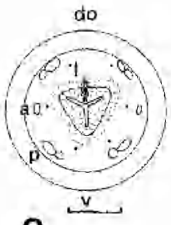

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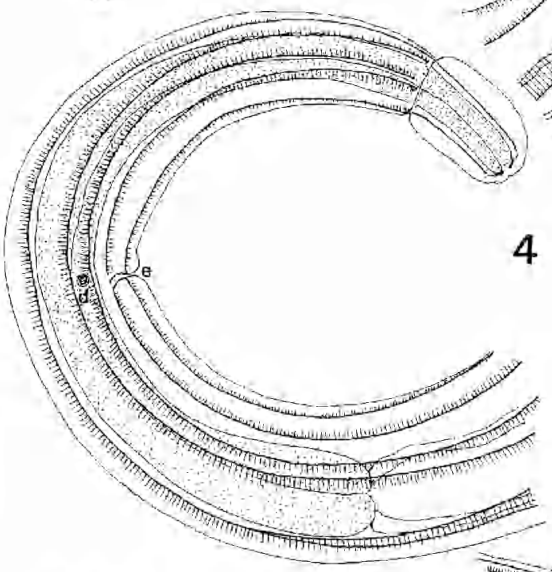

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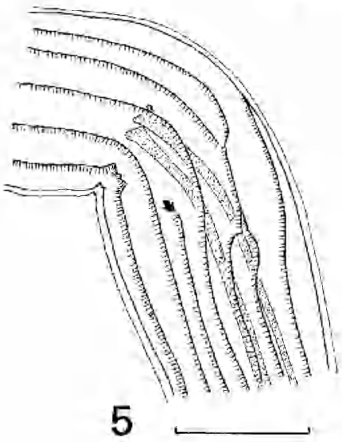

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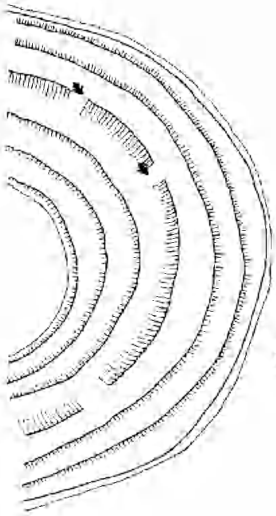

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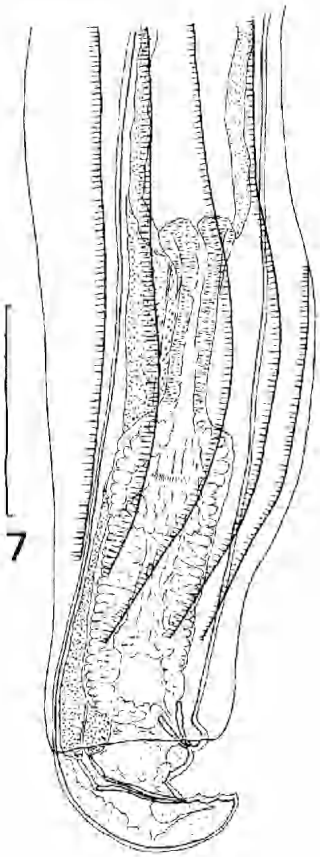

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Description: Small, sinistrally-coiled nematodes, red in colour when live; prominent slightly asymmetrical cephalic vesicle present, buccal capsule vestigial; mouth opening sub-triangular, surrounded by six tiny labial papillae; four double submedian papillae and paired amphids present, external to labial papillae; oesophagus claviform; nerve ring in mid-oesophageal region; deirids dome-shaped, in region of excretory pore.

Synlophe: composed of 14 ridges in mid-body region; axis of orientation from right-ventral field to left dorsal field, at approximately 60° to sagittal axis (Fig. 15); carene, or cuticular swelling present in left dorsal field between ridges 2′ and 4; eight ridges in dorsal field; ridges 1-4 diminishing in size, ridges 5 and 6 larger than 7 and 8; six ridges in dorsal field; ridge 1′ very large, diminishing in size to ridge 6; all ridges arise immediately posterior to cephalic vesicle except for ridges 3,2,1 which arise progressively between vesicle and excretory pore, ridges sometimes interrupted in mid-body region, number and orientation of ridges alters in posterior extremity of body.

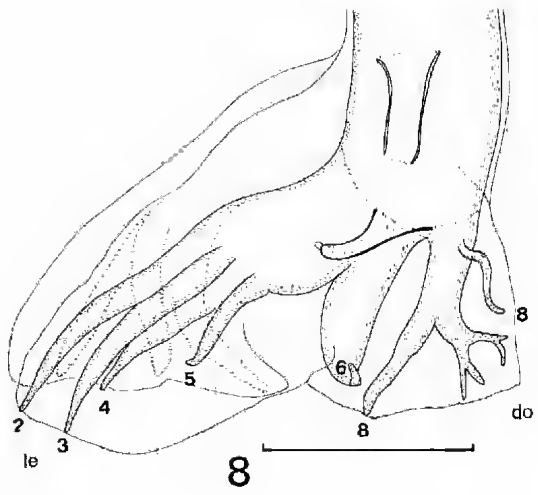
Male. Length 3.3-4.2 (3.7), maximum width 0.10-0.14 (0.11); cephalic vesicle 0.06-0.07 (0.065) long; oesophagus 0.36-0.53 (0.44), nerve ring 0.17 from anterior end; excretory pore 0.25-0.32 (0.28) from anterior end; deirids 0.26-0.32 (0.29) from anterior end; spicules 0.50-0.54 (0.52); gubernaculum 0.05 long. Synlophe: additional ridge arises in right ventral field in region of spicules, between 0.45 and 0.85 from posterior end, immediately anterior to bursa, additional dorsal ridge present, with eight dorsal and eight ventral ridges; ridges reduced in size, orientation barely discernible, ridges of similar size; irregular anastomosing and branching of ridges seen close to bursa. Bursa asymmetrical, right lateral lobe longer than left; dorsal lobe reduced. Dorsal ray with rays 8 arising at different levels; left ray 8 more robust and arising posterior to right; major bifurcation of dorsal ray in posterior third of its length; rays 9 as long as internal rays (10); latter with suggestion of secondary lateral lobe; on left, ray 6 robust, arising close to dorsal trunk, reaching margin of bursa; rays 5 and 4 slender, not reaching margin of bursa, common lateral trunk with prominent bulge at origin of ray 5; rays 3 and 2 elongate, slender, reaching margin of bursa; on right, ray 6 short, slender, arising from lateral trunk; ray 5 slender, reaching margin of bursa; ray 4 extremely robust at base, extremity slender, reaching margin of bursa; rays 3 and 2 slender, reaching margin of bursa. Genital cone prominent, elongate, conical, lightly sclerotised; ventral lobe simple with globoid, non-sclerotised apical appendage; dorsal lobe with two unequal pointed ends, tip surrounded by elongate appendage. Spicules elongate, triquetrous in transverse section; spicule tips dissimilar; tip of left spicule knobbed, with ala arising near tip; tip of right spicule tiny, ala arising at tip; gubernaculum present, lightly sclerotised.

Female. Length 4.6-4.8 (4.7), maximum width in mid-body region 0.12-0.14 (0.13), at posterior extremity 0.14-0.17 (0.15); cephalic vesicle 0.06-0.08 (0.07) long; oesophagus 0.46-0.50 (0.48); nerve ring 0.20 from anterior end; excretory pore 0.26-0.31 (0.29) from anterior end; deirids 0.27-0.31 (0.29) from anterior end; tail 0.03-0.06 (0.05); vulva to posterior end 0.09-0.23 (0.10); egg 0.07-0.08 (0.07) × 0.03-0.05 (0.04). Synlophe: same number of ridges in posterior end of body; ridges become more prominent in region of ovejector, terminate immediately anterior to vulva; ridges of almost equal size, orientation almost lost in posterior region. Posterior extremity of female with swelling of cuticle, variable in shape, often forming sleeve over tip of tail. Tail short, conical, vulva close to anus; monodelphic, ovejector leads to short infundibulum, then into uterus; egg thin-shelled, ellipsoidal.

Discussion

In spite of the fact that the sub-family Nippostrongylinae is cosmopolitan in distribution, and that the type species of *Nippostrongylus*, *N. brasiliensis*, has been widely used as a model in immunological research, few of the species as recognised by Durette-Desset (1970) have been described in detail. Features of the synlophe in the mid-body region have been described for various species by Chabaud & Durette-Desset (1966), Durette-Desset (1969, 1970), Greenberg (1972) and Lichtenfels (1974). Features of the synlophe which might be useful in species separation have been investigated by Lichtenfels (1974) following a detailed examination of the synlophe in laboratory strains of *N. brasiliensis* and limited observations on several additional species. Equally

Figs 1-7. *Nippostrongylus magnus* (Mawson). 1, ♂, cephalic extremity, lateral view, showing asymmetry of cephalic vesicle; 2, apical view of anterior extremity; 3, ♂, anterior end, left side showing origins (arrows) of ridges 1,2 and 3; 4, ♀, anterior end, right side showing origins of ridges at cephalic vesicle; 5, ♂, left view, at level of spicules, 0.5 mm from posterior end, showing origin (arrow) of additional ridge and branching and anastomosing of ridges; 6, mid-body region of ♂, left side, showing discontinuities (arrows) in ridge; 7, female tail, right side, showing sleeve formed by cuticle and termination of ridges. Scale lines: Figs 1,2, 0.01 mm; Figs 3-7, 0.1 mm. Legend: a, amphid; d, deirid; do, dorsal; e, excretory pore; l, labial papilla; p, submedian papilla; v, ventral.



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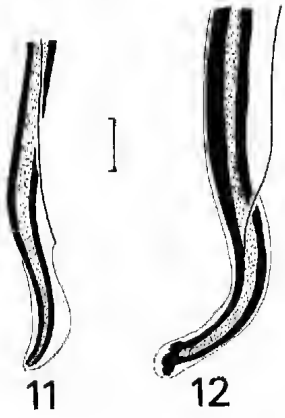

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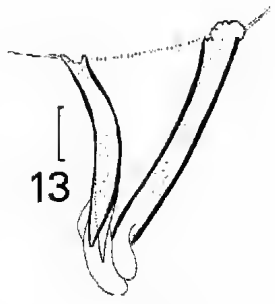

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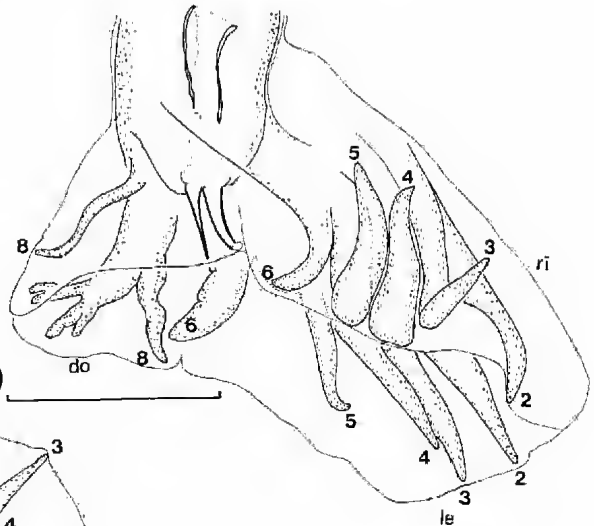

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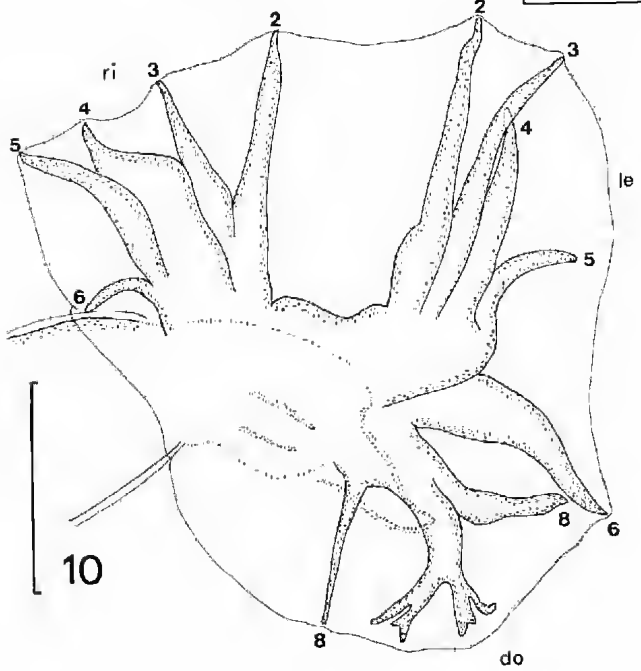

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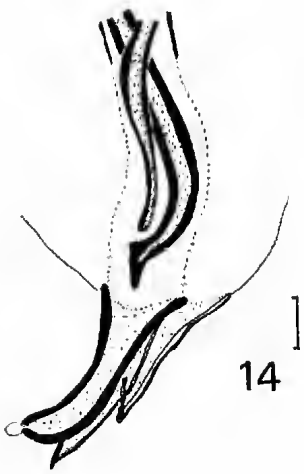

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detailed studies however have not been made on any congeners. Thus, apart from providing a basis for ultrastructural studies currently underway, the detailed description of *N. magnus* is considered valuable as a comparison with studies already carried out on *N. brasiliensis*.

The asymmetry of the bursa has been noted in each congener. The bursa is best studied in apical or ventral views (Durette-Desset 1985), however, in species of *Nippostrongylus* it is extremely difficult to open the bursa, because of its asymmetry. For this reason, left and right lateral views are provided (Figs 8, 9) as well as an apical view (Fig. 10), which was obtained using a live male specimen prior to fixation. The greatest morphological asymmetry occurs in rays 6 and 8, both of which are much larger on the left side of the bursa than on the right, though ray 4 is larger on the right side. Apart from the bursa itself, the spicules and genital cone also exhibit some degree of asymmetry. The tip of the left spicule is much longer and more complex structurally than that of the right spicule, which terminates in a simple point, paralleling the asymmetry of the bursa. Details of the spicule tips have not been provided for congeners except for the tips of the spicules of *N. brasiliensis* (see Mawson 1961). In the case of the genital cone, the (ventral lobe, bearing papilla 0, is symmetrical, while the dorsal lobe, bearing the paired papillae 7 is asymmetrical, with the right papilla longer and hence more posterior than the left (Figs 13, 14). Comparable morphological details are generally lacking for other species, although the genital cone appears to be asymmetrical also in Fig. 1F of *N. rauschi* (see Chabaud & Durette-Desset 1966). Some of these characters may prove useful as generic criteria when described in all species.

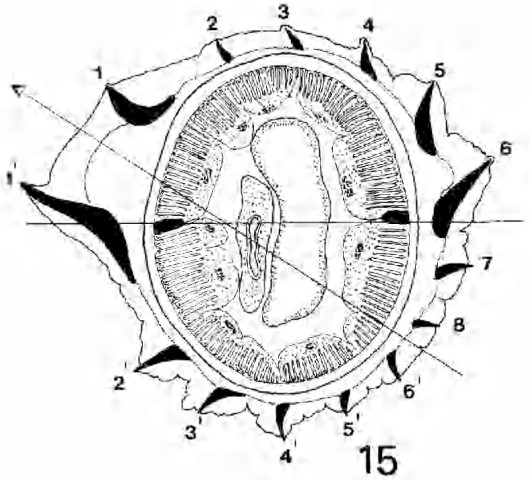
The synlophe is described fully for the first time and confirms the preliminary observations of Durette-Desset (1969) and Lichtenfels (1974). It resembles that of congeners (Chabaud & Durette-Desset 1966; Durette-Desset 1970, 1971; Greenberg 1972) in possessing 14 ridges in the mid-body region with an oblique axis of orientation directed from right-ventral to left-dorsal and a consistent gradient in ridge size. The majority of ridges arise immediately posterior to the cephalic vesicle, with ridges 1,2,3 in the left-dorsal field (ridges 2,3,4 of Lichtenfels 1974) arising immediately anterior to the deirid (1), halfway between deirid and cephalic vesicle (2) and posterior to the vesicle (3). These origins are consistent in males and females and resemble the situation found in *N. brasiliensis* except that in *N. brasiliensis*, ridge 1 arises slightly more posteriorly, at the level of the excretory pore (Lichtenfels 1974). In the posterior region of the male, two additional ridges appear in the left-ventral field, also resembling the arrangement described in *N. brasiliensis* (see Lichtenfels 1974), one about 0.5-0.8 mm from the posterior extremity and a second ridge in the prebursal region. In the posterior region of the female, the number of ridges remains constant, although the ridges become more similar in size and the orientation is more difficult to establish. The extra ridge described in female *N. brasiliensis* by Lichtenfels (1974) is absent in *N. magnus*. Thus the synlophe of *N. magnus* resembles that of *N. brasiliensis* very closely.

The system for numbering ridges employed here differs from that used by Lichtenfels (1974). It attempts to show the axis of orientation and the homology of ridges on either side of the axis. It demonstrates that in both the male and female of *N. magnus*, the asymmetry of ridges and the size gradient are lost in the posterior parts of the body with a symmetrical arrangement of almost equal sized ridges, mostly arranged perpendicular to the body of the nematode. This arrangement would be considered a "hyper-evolved" state in the sense of Durette-Desset (1985). It is of interest that in male *N. magnus*, in the posterior region of the body, not only is there a reduction in size of body ridges and a loss of particular orientation, but also the symmetry of the number of ridges is restored with eight dorsal and eight ventral ridges.

Features of the synlophe of *N. magnus* which might be useful at the specific level are the interruption of ridges in the mid-body region and the irregular branching and anastomosing of ridges in the region of the male bursa, noted by Lichtenfels (1974). In the present study, the interruption of ridges (Fig. 6) occurred in both male and female nematodes, while branching and anastomosing (Fig. 5) was seen in males. Thus Lichtenfels' (1974) observations have been confirmed, but studies of the remaining congeners are required to establish their usefulness.

Lichtenfels (1974) examined specimens of laboratory strains of *N. brasiliensis* adapted to the rat, mouse and hamster and showed that the synlophe was constant, independent of the host species in which the nematode developed. Although much more limited in their extent, the observation that the synlophe of *N. magnus* is identical in specimens from the natural host, *R. fuscipes*, as well as in the laboratory rat, *R. norvegicus*,

Figs 8-14. *Nippostrongylus magnus* (Mawson), male genitalia. 8, bursa, left lateral and dorsal lobes; 9, bursa, right lateral and dorsal lobes; 10, bursa, apical view, left lateral lobe on right hand side; 11, tip of right spicule; 12, tip of left spicule; 13, genital cone, lateral view; 14, genital cone and spicule tips, right ventro-lateral view. Scale lines: Figs 8-10, 0.1 mm; Figs 11-14, 0.01 mm. Figures follow ray numbering system as described by Durette-Desset (1985). Legend: do, dorsal; le, left; ri, right.



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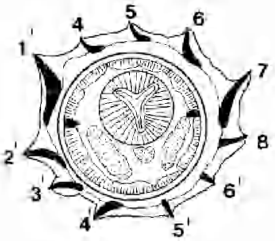

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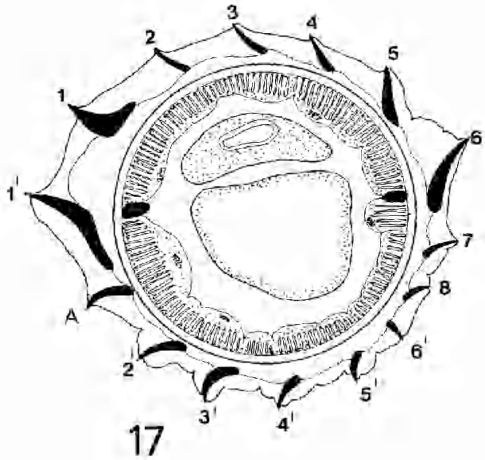

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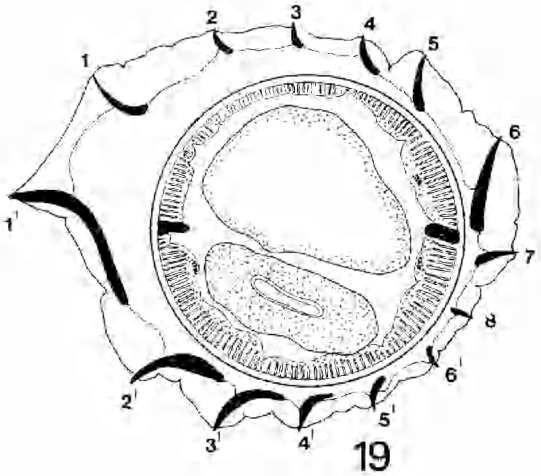

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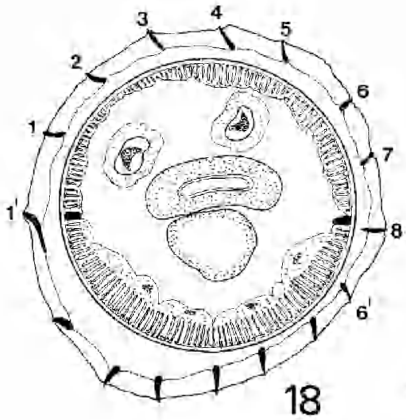

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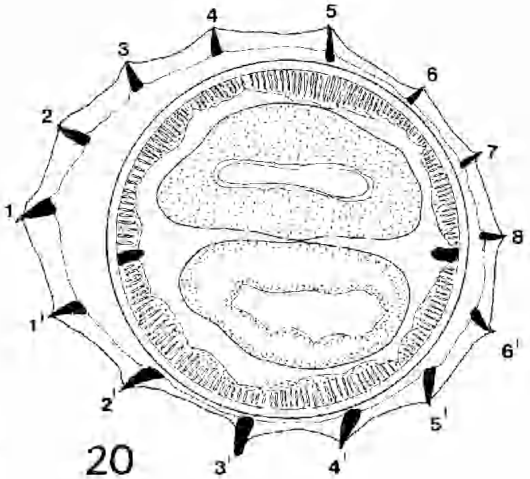

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adds weight to his conclusions on the stability of synlophe characters in different host species.

The affinities of *N. magnus* with congeners have not been fully investigated. Mawson (1961) considered its differentiation from *N. typicus* (both as species of *Austroheligmonema* Mawson, 1961) based on the shape of the spicules, number of ridges and overall size and from *N. brasiliensis*, due to the greater asymmetry in its bursa, and the form of the dorsal ray. Greenberg (1972) provided a comparative table of measurements of all species, but not of other morphological features. Because of the incomplete nature of the descriptions of several species, comparisons are limited to the synlophe and bursal rays. The synlophe is apparently similar in most species of *Nippostrongylus*, but ridge 1 is substantially larger than ridge 2 in *N. magnus*, the male of *N. typicus*, and *N. rauschi*, with the qualification that *N. rauschi* is described as having 14 ridges, but only 13 are illustrated (Chabaud & Durette-Desset 1966, Fig. 2A). In the case of the dorsal ray of *N. magnus*, the asymmetry of rays 8, with a slender right ray arising before a more robust left ray resembles *N. typicus*, but differs from *N. rauschi*, *N. brasiliensis* and *N. djumachani* which have rays 8 arising symmetrically, though with the left ray more robust than the right, and from *N. ryssavyi* in which the left ray 8 arises first and is more slender than the right ray (Erhardova 1959; Mawson 1961; Chabaud & Durette-Desset 1966; Tenora 1969). In *N. witenbergi*, the branching pattern of the dorsal ray resembles that of *N. ryssavyi*, but rays 8 are slender (Greenberg 1972). Thus, *N. magnus* can be differentiated from congeners by several morphological features, in addition to the measurements tabulated by Witenberg (1972), but the features discussed indicate a close relationship with *N. typicus*, also a parasite of endemic Australian rodents.

N. magnus is of biogeographical interest because it is an endemic Australian species occurring in various species of *Rattus* and occasionally in *Melomys cervinipes*. The full host range may be greater than this as a number of endemic rodent species in Australia have not yet been examined for helminth parasites (Mackerras 1958). The endemic species of *Rattus* probably arrived in Australia about one million years ago (Watts & Aslin 1981), hence the morphological differentiation between *N. brasiliensis*/*N. rauschi* and *N. magnus*/*N. typicus* has probably occurred over this same period of time. There are few instances where a time scale can be placed on morphological differentiation between species of parasitic nematodes.

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References

Chabaud, A. G. & Durette-Desset, M.-C. (1966) *Nippostrongylus rauschi* n.sp. Nématode parasite de dermoptères et considérations sur *N. brasiliensis* parasite cosmopolite des rats domestiques. *Ann. parasitol. hum. comp.* **41**, 243-249.

Durette-Desset, M.-C. (1969) Les systèmes d'arêtes cuticulaires chez les nématodes heligmosomes parasites de Muridés australiens. *Ibid.* **44**, 733-747.

_____ (1970) Le genre *Nippostrongylus* Lane, 1923. (Nématode-Heligmosomatidé). *Ibid.* **45**, 815-821.

_____ (1971) Essai de classification des nématodes heligmosomes. Corrélations avec la paléobiogéographie des hôtes. *Mém. Mus. natn. Hist. nat., Paris sér. A. Zoologie*, **69**, 1-126.

_____ (1985) Trichostrongyloid nematodes and their vertebrate hosts: reconstruction of the phylogeny of a parasitic group. *Advan. Parasitol.* **24**, 239-306.

Erhardova, B. (1959) *Oswaldonema ryssavyi* n.sp. und *Viguella chinensis* n.sp. (Nematoda: Heligmosomatidae) bei Chinesischen nagern. *Cesko. parasitol.* **6**, 93-96.

Greenberg, Z. (1972) Helminths of birds and mammals from Israel IV. Helminths from *Nesokia indica* Gray & Hardwicke, 1832 (Rodentia: Muridae). *Israel J. Zool.* **21**, 63-70.

Lichtenfels, J. R. (1974) Number and distribution of ridges in the cuticle of *Nippostrongylus brasiliensis* (Travassos 1914) (Nematoda: Heligmosomatoidea). *J. Parasitol.* **60**, 285-288.

Mawson, P. M. (1961) Trichostrongyles from rodents in Queensland with comments on the genus *Longistriata* (Nematoda: Heligmosomatidae). *Aust. J. Zool.* **9**, 791-826.

Mackerras, M. J. (1958) Catalogue of Australian mammals and their recorded internal parasites. II. Eutheria. *Proc. Linn. Soc. N.S. Wales* **83**, 101-60.

Obendorf, D. L. (1979) The helminth parasites of *Rattus fuscipes* (Waterhouse) from Victoria, including description of two new nematode species. *Aust. J. Zool.* **27**, 867-879.

Tenora, F. (1969) Parasitic nematodes of certain rodents from Afghanistan. *Vestnik Cesko. spol. Zool.* **33**, 174-192.

Watts, C. H. S. & Aslin, H. J. (1981) "The Rodents of Australia" (Angus & Robertson, Australia).

Figs 15-20. *Nippostrongylus magnus* (Mawson), synlophe. 15, male, mid-body region, 1.9 mm from anterior end of 3.3 mm worm with full complement of 14 ridges; arrow indicates axis of orientation of synlophe; 16, male, anterior oesophageal region prior to origin of ridges 2-3; 17, male, 0.85 mm from posterior end showing additional ridge 1A; 18, male, immediately anterior to bursa, showing reduced size of ridges and additional ventral ridges; 19, female, mid-body region, with full complement of 14 ridges; 20, female, 0.80 mm from posterior extremity showing reduction in size of ridges but maintenance of same number of ridges. Scale line: 0.01 mm.